

The invention also provides a method for the identification of an agent that modulates the activity of any of Hu-Asp-1, Hu-Asp2(a), and Hu-Asp2(b). The invention describes methods to test such agents in cell-free assays to which Hu-Asp2 polypeptide is added, as well as methods to test such agents in human or other mammalian cells in which Hu-Asp2 is present.

#### BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

Sequence ID No. 1—Human Asp-1, nucleotide sequence  
 Sequence ID No. 2—Human Asp-1, predicted amino acid sequence  
 Sequence ID No. 3—Human Asp-2(a), nucleotide sequence  
 Sequence ID No. 4—Human Asp-2(a), predicted amino acid sequence  
 Sequence ID No. 5—Human Asp-2(b), nucleotide sequence  
 Sequence ID No. 6—Human Asp-2(b), predicted amino acid sequence  
 Sequence ID No. 7—Murine Asp-2(a), nucleotide sequence  
 Sequence ID No. 8—Murine Asp-2(a), predicted amino acid sequence  
 Sequence ID No. 9—Human APP695, nucleotide sequence  
 Sequence ID No. 10—Human APP695, predicted amino acid sequence  
 Sequence ID No. 11—Human APP695-Sw, nucleotide sequence  
 Sequence ID No. 12—Human APP695-Sw, predicted amino acid sequence  
 Sequence ID No. 13—Human APP695-VF, nucleotide sequence  
 Sequence ID No. 14—Human APP695-VF, predicted amino acid sequence  
 Sequence ID No. 15—Human APP695-KK, nucleotide sequence  
 Sequence ID No. 16—Human APP695-KK, predicted amino acid sequence  
 Sequence ID No. 17—Human APP695-Sw-KK, nucleotide sequence  
 Sequence ID No. 18—Human APP695-Sw-KK, predicted amino acid sequence  
 Sequence ID No. 19—Human APP695-VF-KK, nucleotide sequence  
 Sequence ID No. 20—Human APP695-VF-KK, predicted amino acid sequence  
 Sequence ID No. 21—T7-Human-pro-Asp-2(a)ΔTM, nucleotide sequence  
 Sequence ID No. 22—T7-Human-pro-Asp-2(a)ΔTM, amino acid sequence  
 Sequence ID No. 23—T7-Caspase-Human-pro-Asp-2(a)ΔTM, nucleotide sequence  
 Sequence ID No. 24—T7-Caspase-Human-pro-Asp-2(a)ΔTM, amino acid sequence  
 Sequence ID No. 25—Human-pro-Asp-2(a)ΔTM (low GC), nucleotide sequence  
 Sequence ID No. 26—Human-pro-Asp-2(a)ΔTM, (low GC), amino acid sequence  
 Sequence ID No. 27—T7-Caspase-Caspase 8 cleavage-Human-pro-Asp-2(a)ΔTM, nucleotide sequence  
 Sequence ID No. 28—T7-Caspase-Caspase 8 cleavage-Human-pro-Asp-2(a)ΔTM, amino acid sequence  
 Sequence ID No. 29—Human Asp-2(a)ΔTM, nucleotide sequence  
 Sequence ID No. 30—Human Asp-2(a)ΔTM, amino acid sequence  
 Sequence ID No. 31—Human Asp-2(a)ΔTM(His)<sub>6</sub>, nucleotide sequence  
 Sequence ID No. 32—Human Asp-2(a)ΔTM(His)<sub>6</sub>, amino acid sequence  
 Sequence ID No.s 33-46 are described below in the Detailed Description of the Invention.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 shows the nucleotide (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human Asp1.

Figure 2: Figure 2 shows the nucleotide (SEQ ID NO:3) and predicted amino acid sequence (SEQ ID NO:4) of human Asp2(a).

Figure 3: Figure 3 shows the nucleotide (SEQ ID NO:5) and predicted amino acid sequence (SEQ ID NO:6) of human Asp2(b). The predicted transmembrane domain of Hu-Asp2(b) is enclosed in brackets.

Figure 4: Figure 4 shows the nucleotide (SEQ ID No. 7) and predicted amino acid sequence (SEQ ID No. 8) of murine Asp2(a)

Figure 5: Figure 5 shows the BestFit alignment of the predicted amino acid sequences of Hu-Asp2(a) and murine Asp2(a)

Figure 6: Figure 6 shows the nucleotide (SEQ ID No. 21) and predicted amino acid sequence (SEQ ID No. 22) of T7-Human-pro-Asp-2(a)ΔTM

Figure 7: Figure 7 shows the nucleotide (SEQ ID No. 23) and predicted amino acid sequence (SEQ ID No. 24) of T7-caspase-Human-pro-Asp-2(a)ΔTM

Figure 8: Figure 8 shows the nucleotide (SEQ ID No. 25) and predicted amino acid sequence (SEQ ID No. 26) of Human-pro-Asp-2(a)ΔTM (low GC)

Figure 9: Western blot showing reduction of CTF99 production by HEK125.3 cells transfected with antisense oligomers targeting the Hu-Asp2 Mma

Figure 10: Western blot showing increase in CTF99 production in mouse Neuro-2a cells cotransfected with APP-KK with and without Hu-Asp2 only in those cells cotransfected with Hu-Asp2. A further increase in CTF99 production is seen in cells cotransfected with APP-Sw-KK with and without Hu-Asp2 only in those cells cotransfected with Hu-Asp2

Figure 11: Figure 11 shows the predicted amino acid sequence (SEQ ID No. 30) of Human-Asp2(a)ΔTM

Figure 12: Figure 11 shows the predicted amino acid sequence (SEQ ID No. 30) of Human-Asp2(a)ΔTM(His)<sub>6</sub>

#### DETAILED DESCRIPTION OF THE INVENTION

A few definitions used in this invention follow, most definitions to be used are those that would be used by one ordinarily skilled in the art.

When the  $\beta$  amyloid peptide any peptide resulting from beta secretase cleavage of APP This includes, peptides of 39, 40, 41, 42 and 43 amino acids, extending from the  $\beta$ -

secretase cleavage site to 39, 40, 41, 42 and 43 amino acids.  $\beta$  amyloid peptide also means sequences 1-6, SEQ. ID. NO. 1-6 of US 5,750,349, issued 12 May 1998 (incorporated into this document by reference). A  $\beta$ -secretase cleavage fragment disclosed here is called CTF-99, which extends from  $\beta$ -secretase cleavage site to the carboxy terminus of APP.

When an isoform of APP is discussed then what is meant is any APP polypeptide, including APP variants (including mutations), and APP fragments that exists in humans such as those described in US 5,766,846, col 7, lines 45-67, incorporated into this document by reference and see below.

The term " $\beta$ -amyloid precursor protein" (APP) as used herein is defined as a polypeptide that is encoded by a gene of the same name localized in humans on the long arm of chromosome 21 and that includes " $\beta$ AP - here " $\beta$ -amyloid protein" see above, within its carboxyl third. APP is a glycosylated, single-membrane spanning protein expressed in a wide variety of cells in many mammalian tissues. Examples of specific isotypes of APP which are currently known to exist in humans are the 695-amino acid polypeptide described by Kang et. al. (1987) Nature 325:733-736 which is designated as the "normal" APP; the 751-amino acid polypeptide described by Ponte et al. (1988) Nature 331:525-527 (1988) and Tanzi et al. (1988) Nature 331:528-530; and the 770-amino acid polypeptide described by Kitaguchi et. al. (1988) Nature 331:530-532. Examples of specific variants of APP include point mutation which can differ in both position and phenotype (for review of known variant mutation see Hardy (1992) Nature Genet. 1:233-234). All references cited here incorporated by reference. The term "APP fragments" as used herein refers to fragments of APP other than those which consist solely of  $\beta$ AP or  $\beta$ AP fragments. That is, APP fragments will include amino acid sequences of APP in addition to those which form intact  $\beta$ AP or a fragment of  $\beta$ AP.

When the term "any amino acid" is used, the amino acids referred to are to be selected from the following, three letter and single letter abbreviations - which may also be used, are provided as follows:

Alanine, Ala, A; Arginine, Arg, R; Asparagine, Asn, N; Aspartic acid, Asp, D; Cystein, Cys, C; Glutamine, Gln, Q; L-glutamic Acid, Glu, E; Glycine, Gly, G; Histidine, His, H; Isoleucine, Ile, I; Leucine, Leu, L; Lysine, Lys, K; Methionine, Met, M; Phenylalanine, Phe, F; Proline, Pro, P; Serine, Ser, S; Threonine, Thr, T; Tryptophan, Trp, W; Tyrosine, Tyr, Y; Valine, Val, V; Aspartic acid or Asparagine, Asx, B; Glutamic acid or Glutamine, Glx, Z; Any amino acid, Xaa, X.